REMARKS

Claims 2 and 10 are pending in this application. No new matter has been added. Reexamination and reconsideration of the application are respectfully requested.

A. Compliance with 35 USC 102(b)

The issue is whether the claims are in compliance with 35 USC 102(b) or anticipated by Logothetou-Rella et al., Eur Urol 15: 259 (1988). The rule according to MPEP 2131 is that to anticipate a claim, the reference must teach every element of the claim. Here, the following elements are missing from Logothetou-Rella et al.: 1) feeders, i.e., non-proliferating epithelial cells, described in section 1 below; 2) confluent, described in section 2 below; 3) non-proliferating, described in section 3 below; and 4) normal, described in section 4 below.

1. There is no evidence in **Logothetou-Rella et al**. that some of the cells feed others, whereas feeders are necessary to bladder cell growth according to **Ehmann et al.**

a. In **Logothetou-Rella et al.**, there are no data showing, for example, that conditioned medium stimulates cell proliferation (indicating secreted factors) or that higher plating densities increase cell proliferation (suggesting a cell-cell contact mechanism of growth stimulation). In fact, in case of this cell line, just the factors such as serum in the medium alone may have stimulated cell growth.

b. In **Ehmann et al.**, Fig. 3, the line with open squares, representing bladder cells plated without feeders, indicates no cell multiplication, whereas the line with filled circles, indicating bladder cells plated with feeders, indicates continued multiplication of the bladder cells.

2. Cell proliferation according to **Ehmann et al.** depends on direct contact of the feeder cells with the human bladder cells, whereas cell proliferation according to **Logothetou-Rella et al.**, if it, in fact, is stimulated by other cells in the culture, is probably stimulated by soluble growth factors secreted by those cells into the medium, because bladder cell numbers increase in the absence of confluency, i.e., absence of cell contact.

a. In **Ehmann et al.** cells must be plated at a confluent density for growth to occur, indicating that the growth stimuli are transmitted directly from cell to cell.

• ¶ 0077 with regard to cell contact: "Since soluble growth factors do not appear to be involved in the stimulus to proliferate in the culture systems of the invention, it is likely that the success of this method depends on stimulation by membrane bound growth factors and/or the formation of physical junctions between feeder cells and the epithelial cells."

• ¶ 0089 with regard to plating density: "...the total number of cells was adjusted such that a **confluent monolayer** was achieved with a combination of the human bladder epithelial cells and LA7 cells together."

• Fig. 3: The cells were plated at **~24 x 10^4/cm^2** (calculating from open circles on day 2 in a 35 mm dish).

b. Logothetou-Rella et al. plate cells at a non-confluent density, not a confluent density as argued by the Patent Office on p.3, para.3. Therefore, cells that are probably not in contact with other cells can proliferate. This would indicate that growth is stimulated by a soluble factor in the medium.

• P.260, para. 1: "...by plating **1.1 x 10^4 cells/cm^2** in 25-cm^2 Falcon flasks...", a non-confluent density and 4.4% the density of that in Ehmann, et al.

• P. 260, para. 3 under Results: "...during **the nonconfluent stage**"

• P. 261, Fig. 2 legend: "**Nonconfluent cell culture**"

3. The feeder cells in **Ehmann et al.** are lethally inactivated by radiation and all die as the bladder cells increase in number, leaving only vital bladder cells in the end stage culture. The supposed feeders of **Logothetou-Rella et al.** only senesce. They seem to become part of the end stage culture. There is no evidence that they die to leave only vital bladder cells in the end stage culture.

a. In **Ehmann et al.**, Fig. 3: The line connecting filled circles and representing human bladder cell numbers increases with time to reach almost the total cell number in the dish by day 28. This is described in ¶ 0094: "The human bladder epithelial cells multiplied to displace dying feeder cells until the entire culture became confluent with human bladder epithelial cells".

b. There is no evidence in the **Logothetou-Rella et al.** manuscript that the existing cells provide any growth stimuli to the vital bladder cells, nor is there any evidence that the existing senescing cells actually die, leaving only vital cells.

4. The method of **Ehmann et al.** is observed to support proliferation of bladder cells from any human, whereas the method of **Logothetou-Rella et al.** describes only one cell line obtained by their method. This brings into question the normality of that one cell line.

a. In **Ehmann et al.** "cells from 5 normal bladder tissues adapted easily to tissue culture" (¶ 0094), i.e., bladder tissues from all 5 subjects put into culture proliferated.

b. In **Logothetou-Rella et al.** "A normal bladder epithelial cell line (N cells) was established from normal bladder urothelium...". There is no report of success with tissues from other subjects, nor, to Applicant's knowledge, have there been any reports in the literature by others in these many years since 1988 of successful cultures with their technique. This brings into question the normality of their cells. Even though the chromosome analysis on P. 261 appears to be normal, the keratin staining was not. On P. 261, Immunocytochemistry, "N cells were immunostained for keratin at the granular paranuclear body. The cytoskeleton and projections remained unstained." The cytoskeleton of epithelial cells, however, is composed of keratins, and they project throughout the cytoplasm and into the desmosomes at the cell borders. The entire cytoplasm should have lit up with anti-keratin antibodies if the cells were, in fact, normal epithelial cells. Therefore, one would conjecture that the cell line derived here is not a normal epithelial line, and that is why it proliferated in these culture conditions. (Normal staining of bladder epithelial cells can be seen in **Ehmann et al.** in Fig. 4a and c, where the entire cells are brightly stained.)

In summary, the following elements are missing from the reference: 1) feeders, i.e., non-proliferating epithelial cells; 2) confluent; 3) non-proliferating; and 4) normal. Even if the Patent Office agrees that the reference does not teach a mere one of these elements, the reference fails to anticipate the claims. The conclusion is that the reference does not teach every element of the claims, thus the claims are in compliance with 35 USC 102(b).

CONCLUSION

In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of all outstanding rejections are respectfully requested. Allowance of the claims at an early date is solicited. If any points remain that can be resolved by telephone, the Examiner is invited to contact the undersigned at the below-given telephone number.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 6/26/06

By: _____

Nancy W. Vensko
Registration No. 36,298
Attorney of Record
Customer No. 20,995
(805) 547-5580

AMEND
2694651
061906